

WHAT IS CLAIMED IS:

1. A method of preparing sequences on a substrate comprising the steps of:

- 5 a) exposing a first region of said substrate to an activator to remove a protective group;
- b) exposing at least said first region to a first monomer;
- 10 c) exposing a second region to an activator to remove a protective group; and
- d) exposing at least said second region to a second monomer.

15 2. The method as recited in claim 1 wherein said steps of exposing to an activator use an activator selected from the group consisting of ion beams, electron beams, gamma rays, x-rays, ultra-violet radiation, light, infra-red radiation, microwaves, electric currents, radiowaves, and combinations thereof.

20 3. The method as recited in claim 1 wherein said protective groups are photosensitive protective groups.

25 4. The method as recited in claim 1 wherein said steps of exposing to an activator are steps of applying light to selected regions of said substrate.

30 5. The method as recited in claim 1 wherein said first and the second monomers are amino acids.

35 6. The method as recited in claim 1 further comprising a step of screening sequences on said substrate for affinity with a receptor, said step of screening further comprising the step of exposing said substrate to said receptor and testing for the presence of said receptor in said first and said second region.

01

7. The method as recited in claim 6 wherein said receptor is an antibody.

5 8. The method as recited in claim 1 wherein said substrate is selected from the group consisting of polymerized Langmuir Blodgett film, functionalized glass, germanium, silicon, polymers, (poly)tetrafluoroethylene, polystyrene, gallium arsenide, and combinations thereof.

10 9. The method as recited in claim 1 wherein said protective group is selected from the group consisting of ortho-nitrobenzyl derivatives, 6-nitroveratryloxy-carbonyl, 2-nitrobenzyloxycarbonyl, cinnamoyl derivatives, and mixtures thereof.

15 10. The method as recited in claim 1 wherein said first and second regions each have total areas of less than 1 cm².

20 11. The method as recited in claim 1 wherein said first and second regions each have total areas of between about 1 μm^2 and 10,000 μm^2 .

25 12. The method as recited in claim 4 wherein said light is monochromatic coherent light.

30 13. The method as recited in claim 1 wherein said steps of exposing to an activator are carried out with a solution in contact with said substrate.

35 14. The method as recited in claim 13 wherein said solution further comprises said first or said second monomer.

15. The method as recited in claim 6 wherein said receptor further comprises a marker selected from the group consisting of radioactive markers and fluorescent

markers and wherein said step of testing for the presence of the receptor is a step of detecting said marker.

5 16. The method as recited in claim 1 wherein the steps of exposing to an activator further comprise steps of:

10 a) placing a mask adjacent to said substrate, said mask having substantially transparent regions and substantially opaque regions at a wavelength of light; and

b) illuminating said mask with a light source, said light source producing at least said wavelength of light.

15 17. The method as recited in claim 1 wherein said steps are repeated so as to synthesize 10^3 or more different sequences on said substrate.

20 18. The method as recited in claim 1 wherein said steps are repeated so as to synthesize 10^6 or more different sequences on said substrate.

25 19. A method of synthesizing a plurality of chemical sequences, said chemical sequences comprising at least a first and a second monomer, comprising the steps of:

30 a) at a first region on a substrate having at least a first and a second region, said first and said second region comprising a substrate protective group, activating said first region to remove said substrate protective group in said first region;

35 b) exposing said first monomer to said substrate, said first monomer further comprising a first monomer protective group, said first monomer binding at said first region;

c) activating said second region to remove said substrate protective group in said second region;

d) exposing said second monomer to said substrate, said second monomer further comprising a second monomer protective group, said second monomer binding at said second region;

5 e) activating said first region to remove said first monomer protective group;

f) exposing a third monomer to said substrate, said third monomer binding at said first region to produce a first sequence;

10 g) activating said second region to remove said second monomer protective group; and

h) exposing a fourth monomer to said substrate, said fourth monomer binding at said second region to produce a second sequence, said second sequence
15 different from said first sequence.

20 20. A method of synthesizing a plurality of chemical sequences, said chemical sequences comprising at least a first and a second monomer, comprising the steps of:

a) on a substrate having at least a first and a second region deactivating said first region to provide a first protective group in said first region;

25 b) exposing said first monomer to said substrate, said first monomer binding at said second region;

c) removing said protective group in said first region;

30 d) deactivating said second region to provide a second protective group in said second region;

e) exposing said second monomer to said substrate, said second monomer binding at said first region;

35 f) removing said protective group in said second region;

g) deactivating said first region to provide a protective group in said first region;

h) exposing a third monomer to said substrate, said third monomer binding at said second region to produce a first sequence;

5 i) removing said protective group in said first region; and

j) exposing a fourth monomer to said substrate, said fourth monomer binding at said first region to produce a second sequence, said second sequence different than said first sequence.

10

21. A method of synthesizing at least a first polymer sequence and a second polymer sequence on a substrate, said first polymer sequence having a different monomer sequence from said second polymer sequence, comprising the steps of:

15

a) inserting a first mask between said substrate and an energy source, said mask having first regions and second regions, said first regions permitting passage of energy from said source, said second regions blocking energy from said source;

20

b) directing energy from said source at said substrate, said energy removing a protective group from first portions of said first polymer under said first regions of said first mask;

25

c) exposing a second portion of said first polymer to said substrate to create a first polymer sequence;

d) inserting a second mask between said substrate and said energy source, said second mask having first regions and second regions;

30

e) directing energy from said source at said substrate, said energy removing said protective group under said first regions of said second mask from first portions of said second polymer; and

35

f) exposing a second portion of said second polymer to said substrate, said second portion of said

second polymer binding with said first portion of said second polymer to create a polymer 8second sequence.

5 22. The method as recited in claim 21 wherein said energy is selected from the group consisting of ion beams, electron beams, gamma rays, x-rays, ultra-violet radiation, light, infra-red radiation, microwaves, electric fields, radiowaves, and combinations thereof.

10 23. The method as recited in claim 19 wherein said protective groups are photosensitive protective groups.

15 24. The method as recited in claims 19 or 20 wherein said steps of activating and deactivating are steps of applying light to selected regions of said substrate.

20 25. The method as recited in claims 19 or 20 wherein said first and said second monomers are amino acids.

25 26. The method as recited in claims 19, 20 or 21 further comprising a step of screening said first and said second sequences for affinity with a first receptor, said step of screening further comprising a step of exposing said substrate to said first receptor and testing for the presence of said first receptor.

30 27. The method as recited in claim 26 wherein said step of screening is a step of screening with antibodies.

35 28. The method as recited in claims 19, 20 or 21 wherein said substrate is selected from the group consisting of a polymerized Langmuir Blodgett film, functionalized glass, germanium, silicon, polymers, (poly)tetrafluoroethylene, gallium arsenide, gallium

phosphide, silicon oxide, silicon nitride and combinations thereof.

5 29. The method as recited in claim 19 wherein said protective group, said first monomer protective group, and said second monomer protective group are selected from the group consisting of ortho-nitrobenzyl derivatives, 6-nitroveratryloxycarbonyl, 2-nitrobenzyloxycarbonyl, and mixtures thereof.

10 30. The method as recited in claim 20 wherein said protective group is a cinnamate group.

15 31. The method as recited in claims 19 or 20 wherein said first and second regions each have total areas of less than 1 cm^2 .

20 32. The method as recited in claims 19 or 20 wherein said first and second regions each have total areas of between about $1 \mu\text{m}^2$ and $10,000 \mu\text{m}^2$.

33. The method as recited in claim 24 wherein said light is monochromatic coherent light.

25 34. The method as recited in claim 19 wherein said steps of activating are carried out with a solution in contact with said substrate.

30 35. The method as recited in claim 34 wherein said solution further comprises a monomer.

35 36. The method as recited in claim 26 wherein said receptor further comprises a marker selected from the group consisting of radioactive markers and fluorescent markers and wherein said step of testing for the presence of the receptor is a step of detecting said marker.

37. The method as recited in claims 19 or 20 wherein two of said first, said second, said third, and said fourth monomers are the same monomers.

5 38. The method as recited in claim 21 wherein the step of inserting a second mask is a step of translating said first mask from a first position to a second position.

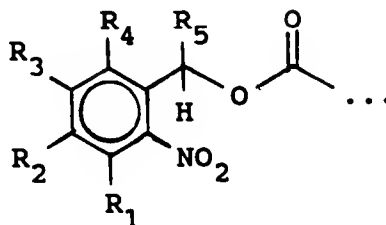
10 39. The method as recited in claim 21 wherein the step of inserting a second mask is a step of rotating said first mask.

15 40. The method as recited in claim 26 further comprising the step of exposing said substrate to a second, labeled receptor, said second, labeled receptor binding at multiple sites on said first receptor.

20 41. The method as recited in claim 40 wherein said first receptor is an antibody of a first animal species and said second receptor is an antibody derived from a second species and directed at said first species.

25 42. The method as recited in claim 19 wherein:
a) said first monomer protective group is removable upon exposure to a first wavelength of light;
b) said second monomer protective group is removable upon exposure to a second wavelength of light;
c) said step of activating said first region
30 to remove said first monomer protective group is a step of exposing substantially all of said substrate to said first wavelength of light; and
d) said step of activating said second region
to remove said second monomer protective group is a step
35 of exposing substantially all of said substrate to said second wavelength of light.

43. A method as recited in claims 19 or 21 wherein said protective groups are of the form:



where R₁ is alkoxy, alkyl, halo, aryl, alkenyl, or hydrogen; R₂ is alkoxy, alkyl, halo, aryl, nitro, or hydrogen; R₃ is alkoxy, alkyl, halo, nitro, aryl, or hydrogen; R₄ is alkoxy, alkyl, hydrogen, aryl, halo, or nitro; and R₅ is alkyl, alkynyl, cyano, alkoxy, hydrogen, halo, aryl, or alkenyl.

44. A method of screening a plurality of amino acid sequences for binding with a receptor comprising the steps of:

- a) on a glass plate having at least a first surface, said at least a first surface comprising a photoprotective material selected from the group consisting of nitroveratryloxy carbonyl and nitrobenzyloxy carbonyl, reacting said at least a first surface with t-butoxycarbonyl for storage, said glass plate substantially transparent to at least ultraviolet light;
- b) exposing said at least a first surface to TFA to remove said t-butoxycarbonyl;
- c) placing said glass plate on a reactor, said reactor comprising a reactor space, said at least a first surface exposed to said reactor space;
- d) placing a mask at a first position on said glass plate, said mask comprising first locations and second locations, said first locations substantially transparent to at least ultraviolet light and said second locations substantially opaque to at least ultraviolet

light, said second locations comprising a light blocking material on a first surface of said mask, said first surface of said mask placed in contact with said glass plate;

5 e) filling said reactor space with a reaction solution;

f) illuminating said mask with at least ultraviolet light, said ultraviolet light removing said photoprotective material from said at least a first surface of said glass plate under said first locations of said mask;

10 g) exposing said first surface to a first amino acid, said first amino acid binding to regions of said at least a first surface from which said photoprotective material was removed, said first amino acid comprising said photoprotective group at a terminus thereof;

h) placing a mask in contact with said glass plate at a second position;

20 i) illuminating said mask with at least ultraviolet light, said ultraviolet light removing said photoprotective material from said at least a first surface of said glass plate under said first locations of said mask;

25 j) exposing said at least a first surface to a second amino acid, said second amino acid binding to regions of said at least a first surface from which said photoprotective material was removed, said second amino acid comprising said photoprotective group at a terminus thereof;

30 k) placing a mask in contact with said glass plate at a third position;

35 l) illuminating said mask with at least ultraviolet light, said ultraviolet light removing said photoprotective material from said at least a first surface of said glass plate under said first locations of said mask;

m) exposing said at least a first surface to a third amino acid, said third amino acid binding to regions of said at least a first surface from which said photoprotective material was removed;

5 n) placing a mask in contact with said glass plate at a fourth position;

10 o) illuminating said mask with at least ultraviolet light, said ultraviolet light removing said photoprotective material from said at least a first surface of said glass plate under said first locations of said mask;

15 p) exposing said at least a first surface to a fourth amino acid, said fourth amino acid binding to regions of said at least a first surface from which said photoprotective material was removed, said at least a first surface comprising at least first, second, third, and fourth amino acid sequences;

20 q) exposing said at least a first surface to an antibody of interest, said antibody of interest binding more strongly to at least one of said first, said second, said third, or said fourth amino acid sequences;

25 r) exposing said at least a first surface to a receptor, said receptor recognizing said antibody of interest and binding at multiple locations thereof, said receptor comprising fluorescein;

s) exposing said at least a first surface to light, said first surface fluorescing in at least a region where said more strongly bound amino acid sequence is located; and

30 t) detecting and recording fluoresced light intensity as a function of location across said at least a first surface.

45. A method of identifying at least one peptide sequence for binding with a receptor comprising the steps of:

5 a) on a substrate having a plurality of polypeptides, each having a photoremovable protective group, irradiating first selected polypeptides to remove said protective group;

10 b) contacting said polypeptides with a first amino acid to create a first sequence, second polypeptides on said substrate comprising a second sequence; and

c) identifying which of said first or said second sequence binds with said receptor.

15 46. The method as recited in claim 45 wherein said step of identifying further comprises a step of detecting the presence of a marker selected from the group consisting of radioactive markers and fluorescent markers in said receptor.

20 47. The method as recited in claim 45 wherein said step of irradiating is a step of masking a light source with a mask, said mask comprising first transparent regions and second opaque regions.

25 48. The method as recited in claim 47 wherein the step of identifying further comprises the steps of:

30 a) exposing a first receptor to said substrate; and
b) exposing a receptor to said first receptor to said substrate, said receptor to said first receptor comprising a marker.

35 49. The method as recited in claim 48 wherein said marker is selected from the group consisting of radioactive markers and fluorescent markers.

50. The method as recited in claim 48 wherein said first receptor is an antibody from a first species and said receptor to said first receptor is an antibody from a second species directed at said first species.

5 51. A method for screening a plurality of polymers for biological activity comprising exposing a receptor to a substrate having said plurality of said polymers on a surface thereof, each of said polymers occupying an area of less than about 1 cm².

10 52. A method for screening as recited in claim 48 wherein said area is less than about 0.1 cm².

15 53. A method as recited in claim 48 wherein said area is less than about 10,000 μm².

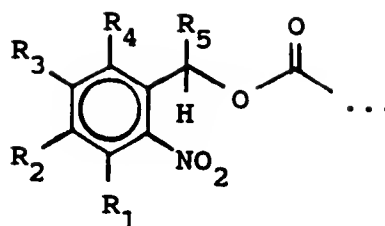
20 54. A method as recited in claim 48 wherein said area is less than about 100 μm².

55. Apparatus for preparation of a plurality of polymers comprising:

25 a) a substrate with a surface, said surface comprising a reactive portion, said reactive portion activated upon exposure to an energy source so as to react with a monomer; and

b) means for selectively protecting and exposing portions of said surface from said energy source.

30 56. Apparatus as recited in claim 55 wherein said reactive portion further comprises a protective group, said protective group of the form:



5

where R₁ is alkoxy, alkyl, halo, aryl, alkenyl, or hydrogen; R₂ is alkoxy, alkyl, halo, aryl, nitro, or hydrogen; R₃ is alkoxy, alkyl, halo, nitro, aryl, or hydrogen; R₄ is alkoxy, alkyl, hydrogen, aryl, halo, or nitro; and R₅ is alkyl, alkynyl, cyano, alkoxy, hydrogen, halo, aryl, or alkenyl.

57. Apparatus as recited in claim 55 wherein said reactive portion further comprises linker molecules.

58. Apparatus as recited in claim 57 wherein said linker molecules are selected from the group consisting of ethylene glycol oligomers, diamines, diacids, amino acids, and combinations thereof.

59. Apparatus as recited in claim 55 wherein said means for selectively protecting further comprises a mask.

60. Apparatus as recited in claim 55 wherein said means for selectively protecting further comprises a light valve.

61. Apparatus as recited in claim 55 wherein said energy source is a light source.

62. Apparatus as recited in claim 55 wherein said reactive portion further comprises a composition selected from the group consisting of nitroveratryloxy carbonyl, nitrobenzyloxy carbonyl, dimethyl-dimethoxybenzyloxy

carbonyl, 5-bromo-7-nitroindoliny1, hydroxy-2-methyl cinnamoyl, and 2-oxymethylene anthraquinone.

5 63. Apparatus for preparation of a substrate having a plurality of amino acid sequences thereon, said apparatus comprising:

- a) a substrate with a surface;
- b) a protective group on said surface, said protective group removable upon exposure to an energy
- 10 source, said energy source selected from the group consisting of light, electron beams, and x-ray radiation;
- c) means for directing said energy source at selected locations on said surface; and
- d) means for exposing amino acids to
- 15 said surface for binding to said surface.

20 64. Apparatus for screening polymers comprising a substrate with a surface, said surface comprising at least two predefined regions, said predefined regions containing different monomer sequences thereon, said predefined regions each occupying an area of less than about 0.1 cm^2 .

25 65. Apparatus as recited in claim 64 wherein said area is less than about 0.01 cm^2 .

66. Apparatus as recited in claim 64 wherein said area is less than $10000 \text{ } \mu\text{m}^2$.

30 67. Apparatus as recited in claim 64 wherein said area is less than about $100 \text{ } \mu\text{m}^2$.

35 68. Apparatus as recited in claims 64, 65, 66, or 67 wherein said monomer sequences are substantially pure within said predefined regions.

69. A substrate for screening for biological activity, said substrate comprising 10^3 or more different ligands on a surface thereof in predefined regions.

5 70. A substrate as recited in claim 69 wherein said substrate comprises 10^4 or more different ligands in predefined regions.

10 71. A substrate as recited in claim 69 wherein said substrate comprises 10^5 or more different ligands in predefined regions.

15 72. A substrate as recited in claim 69 wherein said substrate comprises 10^6 or more different ligands in predefined regions.

73. A substrate as recited in claims 69, 70, 71, or 72 wherein the ligands are peptides.

20 74. A substrate as recited in claim 64 wherein said ligands are substantially pure within said predefined regions.

25 75. Apparatus for screening for biological activity comprising:

30 a) a substrate comprising a plurality of polymer sequences, said polymer sequences attached to a surface of said substrate at known locations on said substrate, each of said sequences occupying an area of less than about 0.1 cm^2 ;

 b) means for exposing said substrate to a receptor, said receptor marked with a fluorescent marker, said receptor binding with at least one of said sequences; and

35 c) means for detecting a location of said fluorescent marker on said substrate.

76. Apparatus for forming a plurality of polymer sequences comprising:

5 a) a substrate, said substrate having at least a first surface and a second surface, said second surface comprising a photoremovable protective material, said substrate substantially transparent to at least light of a first wavelength;

10 b) a reactor body, said reactor body having a mounting surface with a reaction fluid cavity therein, said second surface maintained in a sealed relationship with said mounting surface; and

15 c) a light source for producing light of at least said first wavelength and directed at a surface of said substrate.

77. Apparatus as recited in claim 76 wherein said light source is directed at said first surface.

20 78. Apparatus as recited in claim 76 further comprising a mask, said mask placed between said light source and said first surface, said mask having first regions substantially transparent to said first wavelength of light and second regions substantially opaque to said first wavelength of light.

25 79. Apparatus as recited in claim 76 wherein said cavity comprises a fluid inlet and a fluid outlet, said fluid inlet connected to a pump for flowing reaction fluids through said cavity.

30 80. Apparatus as recited in claim 76 wherein said cavity further comprises a plurality of raised sections.

35 81. Apparatus as recited in claim 78 wherein said mask further comprises a glass plate.

82. Apparatus as recited in claim 81 wherein said opaque regions on said mask comprise chrome.

83. Apparatus as recited in claim 76 wherein at least a portion of said second surface comprises a second photoremovable protective group, said second photoremovable protective group activatable upon exposure to light of a second wavelength.

84. Apparatus as recited in claim 76 further comprising first and second gaskets on said mounting surface and means for maintaining a vacuum between said first and second gaskets.

85. Apparatus as recited in claim 76 wherein said substrate has a thickness of less than 1 mm.

86. Apparatus as recited in claim 76 wherein said substrate has a thickness of less than 0.5 mm.

87. Apparatus as recited in claim 76 wherein said substrate has a thickness of less than 0.05 mm.

88. Apparatus as recited in claim 78 wherein said mask is in direct contact with said substrate.

89. Apparatus as recited in claim 88 wherein opaque regions of said mask are placed in direct contact with said substrate.

90. Apparatus as recited in claim 76 further comprising a liquid crystal light valve for selectively controlling exposure of light to said substrate.

91. Apparatus as recited in claim 76 further comprising a fiber optic faceplate between said light source and said substrate.

92. Apparatus as recited in claim 76 further comprising a molecular microcrystal between said light source and said substrate.

93. Apparatus as recited in claim 76 wherein said cavity comprises light absorptive materials.

94. Apparatus as recited in claim 93 wherein said light absorptive material is N,N-diethylamino 2,4-dinitrobenzene.

95. Apparatus as recited in claim 76 wherein said cavity is filled with a carrier solution.

96. Apparatus as recited in claim 95 wherein said carrier material comprises a material selected from the group of 1-hydroxybenzotriazole, dimethylformamide, diisopropylethylamine, and benzotriazolyl-n-oxy-tris(dimethylamino)phosphoniumhexafluorophosphate.

97. Apparatus as recited in claim 76 wherein said substrate is a fiber optic faceplate.

98. Apparatus for detection of fluorescent marked regions on a substrate comprising:

a) a light source for directing light at a surface of said substrate;

b) a means for detecting light fluoresced from said surface in response to said light source;

c) means for translating said substrate from a first position to a second position; and

d) means for storing fluoresced light intensity as a function of location on said substrate, said means for storing connected to said means for translating and said means for detecting.

99. Apparatus as recited in claim 98 further comprising video display means for displaying light intensity as a function of location on said substrate.

5 100. Apparatus as recited in claim 98 wherein said means for detecting comprises a photomultiplier tube and a photon counter.

10 101. Apparatus as recited in claim 99 wherein said means for directing light further comprises a dichroic mirror, said mirror reflecting light at a wavelength of said light source and passing said fluoresced light.

15 102. Apparatus as recited in claim 100 wherein said light source is a laser light source.

 103. Apparatus as recited in claim 101 wherein said means for storing is a programmed digital computer.

20 104. Apparatus as recited in claim 102 further comprising a microscope, said light source directed at said substrate through said microscope, said means for detecting receiving light from said microscope.

25

add
A4

add
B1

add
73

30

35